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*A New Rapid Gas Chromatographic Method for Ethylene, Respirational, and
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A New Rapid Gas Chromatographic Method for Ethylene, Respirational, and Senescent Gaseous Production of Climacteric Fruits Stored in Prolonged Low Temperature

Muhammad Makky, Konstantinos A. Paschalidis, and Peeyush Soni

Abstract—Gas chromatography (GC) with flame ionization detector (FID) and HP-PLOT Q Column (530 μ m \times 40 μ m \times 30m) was used to determine the concentration of C₂H₄, CO₂, and H₂S from climacteric fruits that kept at 5°C for 7 days under low light condition. Fruits are stored in air-tight jars, and upon measurement, brought onto 20°C room temperature for 60 minutes. One ml gas sample from the upper atmosphere inside the jar is measured by GC. To find the best method for rapid GC analysis, the combination of temperature and pressure in the inlet, FID, and Oven, as well as gas (air, fuel, and carrier) flow and velocity, of the GC need to be sought until the least running time is achieved, while maintaining the accuracy and sensitivity of the results. In this study, the optimum method reduced the analysis time to 8 minutes by setting the pre and post injection time to 0min with air gap of 0.2 μ L, and using Helium as the carrier gas, while maintaining isothermal (60°C) and isobaric (6.8145psi) condition in the inlet, and kept total gas flow at 18ml min⁻¹. Moreover, the sample needs to be split with 2:1 ratio and 10ml min⁻¹ flow, and the inlet flow is maintained at 5ml min⁻¹ with average velocity of 46.974cm sec⁻¹, and 1.0644min holdup time. In order to obtain minimum run times for the analysis, initial FID temperature is set to 250 °C, and the fuel gas flow is set to 30ml min⁻¹. The air flow set to optimum (400ml min⁻¹), and the makeup flow set at 10 ml min⁻¹. Furthermore, the GC oven temperature initially set to 60 °C, maintained for 2 min, then heated (30°C min⁻¹) until reaching 240 °C. This method could detect as little as 154, 393, and 236 ppb of C₂H₄, CO₂, and H₂S respectively.

Keywords—Gas Chromatography, Flame Ionization Detector, Climacteric, Senescence

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I. INTRODUCTION

FRUITS, vegetables, and nuts are come in many forms, but notably can be distinguished into two different groups according to its behavior upon ripening; climacteric and non-climacteric. Fruits that belong to non-climacteric group do not continue their ripening process after harvest, and only give off a very low small traceable ethylene when stored [1]. On the other hand, the climacteric fruits were characterized by its burst of ethylene production and cell respiration, associated with its ripening process after being harvested [2]. Some examples of climacteric fruits are apples, bananas, melons, apricots, and most notably, tomatoes. Climacteric process on fruits implies its finalizing of maturation process, and the onset of senescence, which defined by sudden rise of respiration, and subsequently returned to the normal stage. This event can be observed physiologically through fruits color change, as a sign for its edible ripeness peak, best flavor, taste, and texture. Afterward, fruits will start to decay, indicating by cell disintegrations and degrading appearance.

To understand the climacteric process on fruits, many studies had been undertaken in the recent years. Most researchers were focused on fruits ethylene production, which was closely correlated with ripening in many fruits. The most popular and the best apparatus used by researchers to measure the ethylene production in fruits, among other was gas chromatography (GC) [3]. GC had been used as early as 1947, and since then had become standard technique for analyzing and identifying gases chemistry and compounds through separation, vaporization, purification, without necessity of decomposition [4].

Gas chromatography comes in many types and variations. Nonetheless, its main components and the working principle of this machine generally remain unchanged. This included the carrier gas, flow controller, injector port, column, column oven, detector and recorder [5] The sample gas to be analyzed usually drawn from the head space container using gas tight syringe (1 ml) [6], then injected into the GC for analysis, by comparing it with a standard [7].

Earlier last year, one of the main manufacturers of the GC

machines, Agilent technologies, had just introduced their latest edition of the gas chromatography machine, the 7890B GC system. This new model has proven to exceed the needs of current required performance and offer reliability, accuracy, and precision upon measurements. The system offered integrated intelligence built on and advanced features to perform measurements in much less time, while maintained its reliability and high level of performance [8]. Since this machine offered new features and capabilities, and could be utilized to assess the progress of respirational process and ethylene production in climacteric fruits, it is necessary to setup the most appropriate methods for this machine to perform the most rapid analysis while delivering the most accurate and sensitive results.

The objective of this study was to setup the optimum method for gas chromatography analyses for climacteric fruits effluent (C_2H_4 , CO_2 , and H_2S) concentration using Agilent 7890b GC system with flame ionization detector (FID) and HP-PLOT Q Column ($530\ \mu m \times 40\ \mu m \times 30\ m$).

The results of this study will provide a new standard or reference that will be used by many other researchers for measuring the ethylene and other gaseous effluent from the respiration process of climacteric fruit, and can be correlated to the fruits maturity and shelf life.

II. MATERIALS AND METHODS

A. Fruits Samples

For this study, seven varieties of climacteric fruits, three cultivars of apples, two cultivars of tomatoes, pear, and banana were purchased from Sindos local department store, in Thessaloniki, Greece. The apples cultivars constitute of "Grand Smith", "Golden", and "Starking", all with uniform ripeness, shape and size. The tomato cultivars comprised of "Dramas Extra", and "Cherry", while the pear were selected from one variety ("Krystal") of different ripeness, green and yellow. As for the banana, the selected cultivar was "Chiquita" being the only available in the market. All of the fruits, except for the green "Krystal" pear, were at the peak of their edible ripeness, and ready to be consumed. This means that the fruits were at the best condition of their flavor, tastes, and textures. All samples were purchased in the same time. The fruits selection was based on the market availability at that time.

B. Experiment Setup

All samples were cleaned and labeled, and then each fruit was weighed using analytical balance (ALS 220-4, KERN & Sohn, Germany) to record its initial weight. Air-tight glass-jar containers were prepared to keep the samples (Fig. 1). The caps of the containers were drilled, 1 mm in diameter, to enable head space gas sampling. A rubber sealed was put to cover the hole to prevent the gas inside the container leaked. The samples were put in pair in different jars, according to their varieties. Each container contains around 200 gram of samples. The jars are closed, and put into dark cooling room with $5^\circ C$ temperature for one week. Upon measurements, the

jars were put into normal temperature room ($20^\circ C$) for 1 hour, then 1 ml of headspace gas from each container were withdrawn using air tight syringes and injected into the gas chromatogram (Agilent GC 7890B, Agilent Tech. USA). For the experiment, a complete randomized block design was used to carry out the treatments with three replications.

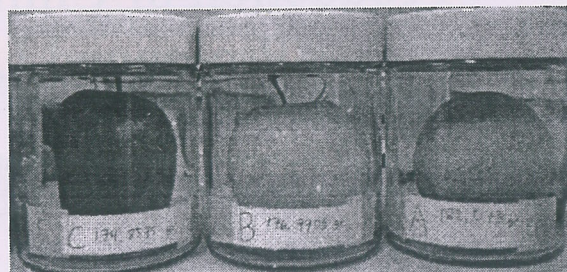


Fig. 1 The fruits samples were put into air-tight glass-jars and then stored in $5^\circ C$ dark room for 7 days.

C. Gas Chromatogram

For the gas chromatograph (GC) setup, the machine was installed with a flame ionization detector (FID), equipped with HP-PLOT Q Column model 19095P-Q04E, with diameter, length and film thickness of $530\ \mu m$, $30\ m$, and $40\ \mu m$ respectively. The column void time is set to 1.057 min with permissible temperature range of $-60^\circ C$ to $250^\circ C$. The GC Syringe sampler (SS) is set to $10\ \mu L$ with injection volume of $1\ \mu L$. The solvent A, B and sample wash volume in the SS are set at $8\ \mu L$, six sample pumps are all activated to channel solvent wash draw, and dispense speed of $300\ \mu L\ min^{-1}$ and $3000\ \mu L\ min^{-1}$ respectively. This enable sample wash draw speed to be set to $300\ \mu L\ min^{-1}$, while the sample wash dispense speed is set at $3000\ \mu L\ min^{-1}$, and injection dispense speed of $6000\ \mu L\ min^{-1}$. Dwell time of pre injection and post injection time are both set to 0 min, as well as the viscosity delay. The standard Injection Type is used with L1 air gap of $0.2\ \mu L$ and sample overlap is disable. The machine front SS inlet use Helium gas as carrier gas, with split mode. The inlet temperature is set to $60^\circ C$ and the inlet pressure maintained at 6.8145 psi, resulting a total gas flow of $18\ ml\ min^{-1}$. The Septum purge flow turned on at $3\ ml\ min^{-1}$ and gas saver mode is activated at $15\ ml\ min^{-1}$ after 2 min. The sample split ratio is set to 2:1 with the split flow at $10\ ml\ min^{-1}$, using the $990\ \mu L$ liner (Agilent 19251-60540), a split, straight liner with glass wool. The column is unlocked, the sample were injected to front SS Inlet sampler with Helium gas as carrier, and channeled out to a front detector FID. The initial GC inlet temperature is set to $60^\circ C$ with 6.8145 psi pressure. The inlet flow maintained at $5\ ml\ min^{-1}$ and average velocity of $46.974\ cm\ sec^{-1}$. The inlet holdup time is set to 1.0644 min, with activated flow program at $5\ ml\ min^{-1}$ for 0 min, and 9 min of run time. The GC front detector FID is heated to $250^\circ C$, and the fuel gas (Hydrogen) flow set to $30\ ml\ min^{-1}$ to maintain the flame. The air flow set to optimum at $400\ ml\ min^{-1}$ and the makeup flow set at $10\ ml\ min^{-1}$. The correction of carrier gas flow does not affect makeup or fuel flow in the system. The

front signals detection is set with data rate sample of 20 Hz. The GC Oven initially set to 60 °C and maintained for 2 min. after the temperature reach equilibrium for 0.5 min, then the oven is heated with the rate of 30 °C.min⁻¹ until it reached 240 °C, then the temperature in maintained for 1 min. The oven max temperature cannot exceed 250 °C, while total GC run time is approximately 9 min. All GC measurement results were prepared using the Agilent Integrated GC Software [9].

D. Statistical Analyses

Descriptive statistical analyses by means of Analysis of variance (ANOVA) are used to analyze the differences between samples variety as well as their variation among and between groups. The data pertaining to climacteric gases effluent from the fruits were subjected to statistical analysis using analysis of variance (ANOVA) according to Fischer's. The level of significance used in F test and t test was set at $p \leq 0.05$. Critical difference values were calculated wherever the 'F' test was found significant.

III. RESULTS AND DISCUSSION

The GC was one of many chromatographic techniques that can be utilized to analyze many organic material and compound by using separation method of volatile chemical. The common arrangement of a GC constitute of a flowing mobile phase, an injection port, a separation column containing the stationary phase, a detector, and a data recording system [10]. The samples can be dispartate through their dissimilarity upon breaking-up, between the carrier gases, inside the column. The gas could either be in the mobile or stationary phase. The common carrier gases used as mobile phases were inert gases such as helium. The injection port constitutes of rubber sealed port or septum, through which a syringe needle is inserted to inject the sample gases [10]. The temperature in the injection port usually was maintained much higher than sample mixture the boiling point. Since the temperature greatly influenced the volatile compound partition, the separation column usually made from certain material that can distribute the temperature evenly when placed in thermal-controlled heating oven.

To accurately identified each volatile compound in the samples, the column temperature is set to low (approximately 30°C) in the initial phase, and over the time the temperature was steadily increased until it reached the highest compound boiling point (approximately 250°C). This method will enable assessment of wide range of volatile compound from the samples. The column used in this study, the HP-PLOT Q Column model 19095P-Q04E, was made from special material suitable for analytical work in chemistry. The column had Low Thermal Mass (LTM) Modules, made of stainless steel, aluminum or polyimide coated fused silica, and contains silicon polymers, polyethylene glycol polymers, silicon and polyethylene glycol polymers, organic porous layer open tubing (PLOT) or inorganic porous layer open tubing (PLOT). The GC column is wrapped with Nextel fiber (Alumino-boro-silicate fibers), organic sizing, and wiring, and then packaged

within a sheet metal case. The small amount of the sample (1 ml) to be analyzed was usually the head space gases of organic material put in enclosed chamber for one hour, in our case were climacteric fruits.

As the carrier gas (helium) flows through the injector and pushes the gas sample into the GC column, the separation of the volatile components takes place, and delivered onto the detector. Different compounds in the mixture will reach the detector at varying times based on its behavior differences when it was partitioned in the mobile and stationary phases. This difference was referred as the retention time. The sensor on the detector will send signals to the data recorder in the machine and display the results in graphs or tables. The calculation of the compounds concentration can be correlated by the peak area of the chart, and generally proportional to the number of molecules reached the sensor.

The essential data obtained from GC measurements were displayed by peaks in charts, indicating their retention time (in minutes), peak's height and width. Identification of each compound in the mixture and its quantity could be made through calculation of its peak area under each curve, by multiplying the half of the peak's width and its height. The data will be then compared to a known standard.

In this study, three known standards were used to identified three compounds from the samples, namely C₂H₄ (ethylene), CO₂ (carbon dioxide), and H₂S (hydrogen sulfide). The results were shown in Fig. 2. The results showed that most of the fruits had already entered their senescence process (Fig. 2a – 2f), indicating by the considerable peak of H₂S. Only banana (Fig. 2h) and red-ripe pear (Fig. 2g) were yet to enter this phase. The peaks also showed that the respiration process of all fruits was at much higher rate compared to their ethylene production. This conclusion could be drawn by comparing the peaks of CO₂ (the first peak) and C₂H₄ (the second peak). Nonetheless, the charts results could only delivered qualitative outcome.

The quantitative analyses of the results were performed by comparing the peak area of each compound with the peak area of the known standards (1.25 ppm of CO₂, C₂H₄, and H₂S). The area then correlated to the standard gas concentration (1.25 ppm) to calculate each compound concentration in the gas sample. Furthermore, by considering the fruits sample weight (in kg), and their storage time (in hours), the rate of each compound generated by the fruits was known, and could be calculated accordingly.

The results (Fig. 3) showed that two types of apple ("Golden" and "Starking") produced significantly higher C₂H₄ compared to other fruits, despite being stored at low temperature (5°C) for the same period (7 days). This gave the indication that the climacteric process in both fruits was still in progress, and storing the fruits in low temperature did not significantly affect the process. On the other hand, all other fruits, have much lower C₂H₄ production upon the storing (Fig. 3a), and suggested that low temperature and duration of the storage affected their climacteric process. The respiration

of the fruits under consideration was assessed based on their CO₂ production (Fig. 3b). On this analysis, only "golden" apple stand among other fruits, and had considerably higher respiration rate compared to other samples. In this measurement, the CO₂ components from apple "Starking" headspace sample gas was unable to be detected by the GC, suggesting that either the compound did not exist or the fruit has entered its senescence phase. Tomato ("Cherry" and "Drama Extra"), the red-ripe pear ("Crystal") and "Grand Smith" apple have similarly CO₂ production rate in this study.

Another component that detected from the gas sample produced by the fruits stored inside closed chamber was H₂S (Fig. 3c). The headspace gas sample drawn from the "Golden" apple has shown the highest concentration of H₂S, followed by apple "Starking" and green-mature pear "Crystal". The gas sample from banana and red-ripe pear appeared to have a very low or insignificant amount of H₂S. The results of this experiment were presented on detail in Table. I.

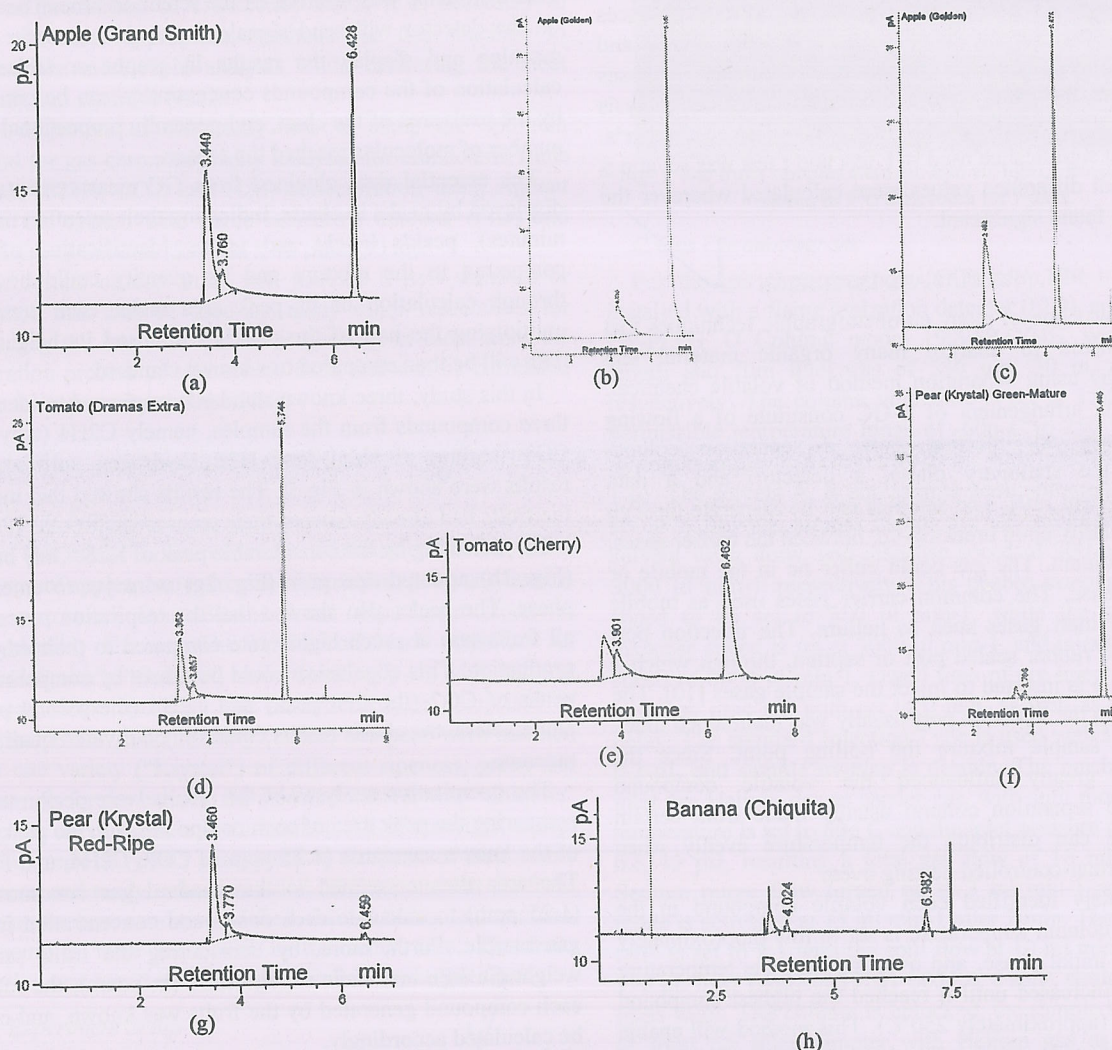


Fig. 2 The chromatogram results of the fruits samples, put into closed chamber for 1 hour at 20°C, then 1 ml of headspace gas was drawn by the syringe and injected in to the GC. The charts showed the peaks of different compounds from the climacteric and respiration process of (a) apple "Grand Smith", (b) apple "Golden", (c) apple "Starking", (d) tomato "Drama Extra", (e) tomato "Cherry", (f) pear "Krystal" (green-mature), (g) pear "Krystal" (red-ripe), and (h) banana "Chiquita". The peaks represent CO₂, C₂H₄, and H₂S in chronological order.

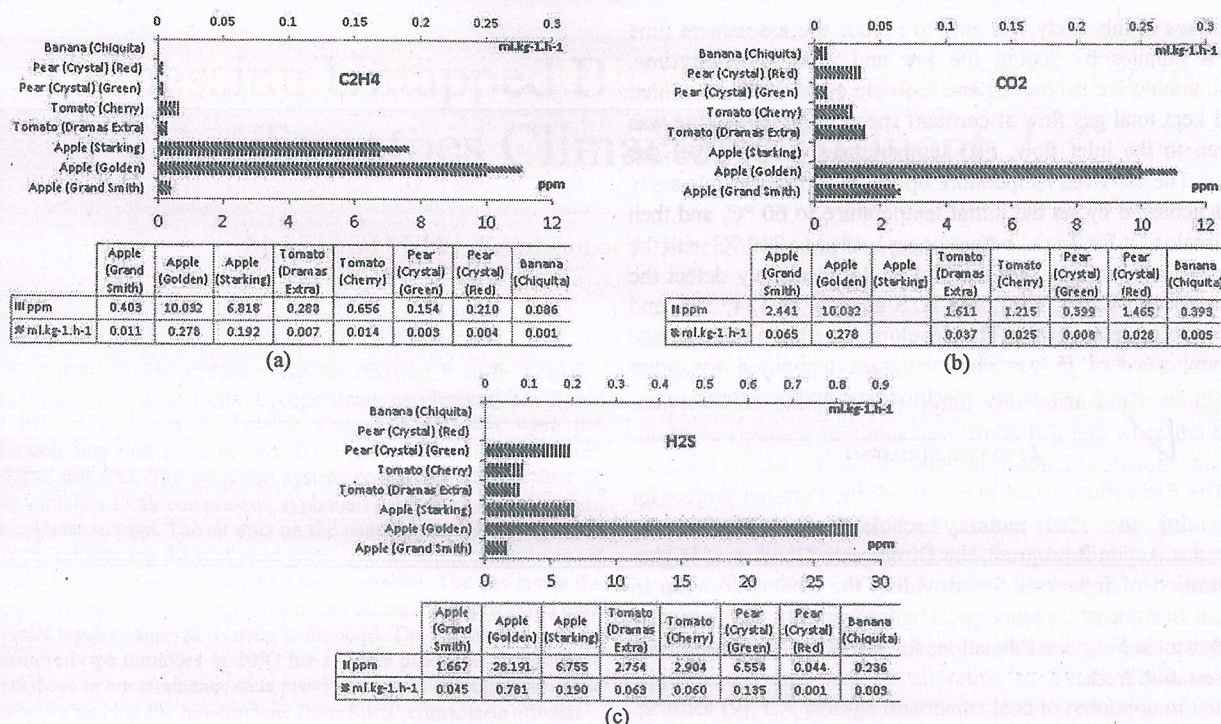


Fig. 3 Quantification analyses results from the samples, representing three main components in the gas samples taken from the headspace of each fruit closed-container, constituting (a) ethylene (C2H4), (b) carbon dioxide (CO2), and (c) hydrogen sulfide (H2S).

TABLE I
THE CONCENTRATIONS AND PRODUCTIONS RATE OF THREE MAIN GAS COMPONENT (C2H4, CO2, AND H2S)
MEASURED FROM EACH FRUIT SAMPLE CLIMACTERIC AND RESPIRATION PROCESSES

Samples ^z	Apple (Grand Smith)	Apple (Golden)	Apple (Starking)	Tomato (Dramas Extra)	Tomato (Cherry)	Pear (Crystal) (Green)	Pear (Crystal) (Red)	Banana (Chiquita)
Weight (gr)	182.073a	176.771a	174.858a	206.228ab	222.31ab	224.328ab	239.325ab	318.263b
C2H4 RT	3.759a	4.567b	3.481a	3.656a	3.901a	3.766a	3.768a	4.024b
Width (min)	0.112	0.318	0.275	0.115	0.152	0.068	0.1	0.052
pA (high)	0.87	3.679	5.907	6.542	0.997	0.576	0.457	0.439
pA (area)	7.092	176.361	119.858	5.059	11.535	2.708	3.699	1.508
ppm	0.403	10.032	6.818	0.288	0.656	0.154	0.21	0.086
Rate*	0.011b	0.278b	0.192b	0.007a	0.014a	0.003a	0.004a	0.001a
CO2 RT	3.439	4.334		3.352	3.608	3.434	3.46	3.652
Width (min)	0.149	0.202		0.131	0.175	0.112	0.125	0.062
pA (high)	4.525	5.568		3.146	1.628	0.887	3.061	1.664
pA (area)	42.903	176.361		28.327	21.351	7.019	25.749	6.903
ppm	2.441	10.032		1.611	1.215	0.399	1.465	0.393
Rate*	0.065ab	0.278b		0.037ab	0.025ab	0.008a	0.028ab	0.005a
H2S RT	6.428	6.956	6.474	5.744	6.462	6.446	6.461	6.982
Width (min)	0.057	0.13	0.087	0.056	0.175	0.085	0.051	0.072
pA (high)	8.063	52.614	19.708	13.717	3.942	21.971	0.201	0.887
pA (area)	29.313	495.587	118.752	48.581	51.043	115.219	0.776	4.153
ppm	1.667	28.191	6.755	2.764	2.904	6.554	0.044	0.236
Rate*	0.045a	0.781b	0.19ab	0.063a	0.06a	0.135ab	0.001a	0.003a

* Rate in ml.kg⁻¹.h⁻¹

^z Means followed by the same letter within rows are non-significant at P = 0.05 by Duncan's multiple range tests.

IV. CONCLUSIONS

In this study an optimum method for Gas chromatography analyses with flame ionization detector (FID) and HP-PLOT Q Column (530μm×40μm×30m) had been determined to assess the concentration of C2H4, CO2, and H2S produced by

climacteric and respiration process of the fruit samples that being kept at 5°C for 7 days under low light condition. The measurements could provide an insight of the fruits ripening progression as well as their senescence commencement. Three main components (C2H4, CO2, and H2S) from the gas sample drawn from the fruits' enclosed camber were assessed qualitatively and quantitatively. The optimum method of GC

analyses in this study was able to reduce the assessment time to 8 minutes by setting the pre and post injection time, maintaining the isothermal and isobaric condition in the inlet, and kept total gas flow at constant speed. Another setting was given to the inlet flow, FID temperatures, and the fuel gas flow. The GC oven temperature optimum setting for this assay was achieved by set the initial temperature to 60 °C, and then maintained it for 2 min, before being heated to 240 °C with the rate of 30°C min⁻¹. This method could accurately detect the presences of C₂H₄, CO₂, and H₂S as little as 154, 393, and 236 ppb of respectively. The developed methods will provide a new standard in gas chromatogram technique for other researches using similar GC machine type and peripheral.

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